Current Trends in Antifertility Vaccine Research

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We review the major advances that have recently occurred in the area of antifertility vaccines by examining the immunogenic potential of gamete, embryonic and placental antigens. In human trials using β-human chorionic gonadotropin coupled with tetanus toxoid as the immunogen, the major problems with antifertility vaccines relate to specificity and maintaining an adequate antibody titer to disrupt gestation. Possible complications include cross-reaction with other body tissues, immune complex deposition, cytotoxicity, impaired immunologic tumor surveillance and nonreversibility.

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Although a number of methods are available today for contraception, none of them are absolutely safe, free from discomfort and universally acceptable. Thus, research continues toward developing a more effective means of fertility regulation. Among these new approaches, active immunization against one or more antigens involved in the reproductive process bears particular promise because it would preclude the disadvantages associated with a daily oral drug regimen.

Ideally, an antifertility vaccine should have the following properties: reasonable titers of antibodies to the immunogen should develop in all recipients; the immunity should persist for a reasonable amount of time, say 6 to 18 months, so that numerous booster injections are not required; the antifertility effect should be reversible; noxious adjuvants such as Freund's complete adjuvant should be avoided; antibodies produced should be specific to the reproductive process without cross-reacting with other body components, and there should be no serious side effects (Table 1).

A number of factors governs the specificity of the immune response to a given antigen. These include the type of antigen, method of presentation, the route of administration and the adjuvant used. Several other factors affect the duration of immunity. These include the persistence of the antigen in vivo, clonal expansion, T- and B-cell cooperation and the production of memory lymphocytes.

In this review, we consider immunologic fertility control in terms of three broad classes of antigens: gamete, embryonic and placental. We focus on active rather than passive immunization because the former method is more efficacious and amenable to large-scale use.

Gamete Antigens

Zona Pellucida

The zona pellucida, an acellular gelatinous layer, surrounds the mammalian oocyte and preimplantation embryo. The zona pellucida prevents polyspermia and blocks penetration of sperm by other species.[1,2] This layer breaks away just before implantation either by a hatching mechanism involving embryo pulsation, by uterine proteolytic activity or by a combination of both.[3] The following features make the zona pellucida an attractive target for immunologic contraception: it is a unique structure, immunization could prevent conception and therefore not act as an abortifacient and there may be as many as four antigenic sites on this structure that might promote specificity and simplicity in antibody production.[4]

The zona-specific antigens develop at the stage of the secondary follicle, persist throughout the ovulation stage and are intact when the sperm-ovum interaction occurs.[5] These antigens are also detectable on the zonae of embryos at the morula and blastocyst stages and are retained until zona shedding during implantation.[6] Thus there is ample opportunity for specific circulating antibodies to bind with zona antigens. Antibodies raised against zona

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ABBREVIATIONS USED IN TEXT
DS_5-hCG-β-hem = a highly specific derivative of β-human chorionic gonadotropin
FSH = follicle-stimulating hormone
hCG = human chorionic gonadotropin
β-hCG-TT = β-hCG coupled with tetanus toxoid
hPL = human placental lactogen
LDH-C_4 = an isoenzyme of lactate dehydrogenase
LH = luteinizing hormone
OLHβ = β-subunit of ovine luteinizing hormone
PP_5 = placental protein-5
SP_1 = Schwangershaft's protein-1

antigens can inhibit fertility, presumably by preventing sperm attachment and passage through the zona.[7] Although zona pellucida antigens are tissue-specific, they are not species-specific; it has been shown that human and porcine zonae (and other species) contain an immunologically similar cross-reacting antigen.[8] Antiserum produced against a purified 60,000 molecular weight component isolated from porcine zonae inhibited in vitro adherence of homologous sperm to antiserum-pretreated human and monkey zonae.[9] Thus the porcine zona pellucida may serve as an antigenic source for the development of a human contraceptive vaccine. Its efficacy would depend on whether sufficiently high antibody titers could be achieved and whether tissue specificity could be assured.

Spermatozoa

In most research in this area two sperm-specific antigens have been used: Lactate dehydrogenase (LDH-C_4) or acrosin. LDH-C_4 (formerly called LDH-X) is an isoenzyme that is specific to the acrosome of spermatozoa. Mouse LDH-C_4 has been isolated in pure form and antibody to it cross-reacts with the human sperm enzyme.[10] Anti-LDH-C_4 causes reduced fertility in rabbits.[11] More promising results were obtained in baboons that were immunized with both human and murine LDH-C_4.[12] Seven of eight matings subsequent to immunization were infertile and the one pregnancy that occurred was in an animal with undetectable antibody titers.

Acrosin is a protease also present in the acrosome. It has been implicated as the enzyme responsible for penetration of the zona pellucida by spermatozoa, and may play a role in the acrosome reaction.[13,14] Studies have shown that acrosin inhibitors can prevent fertilization of the ovum.[15-17] Additional work has shown that immunization of ewes with partially purified ram acrosin reduced but did not eliminate fertility.[18] Because a single ejaculation introduces millions of spermatozoa into the female reproductive tract and only one sperm is required for fertilization, it may be difficult to achieve sufficiently high antibody titers in the oviductal fluid to ensure neutralization of every sperm. Studies in this area to date have shown only reduced fertility or incomplete sterility.

Embryonic Surface Antigens

Several investigators have studied immunization against embryonic cell surface antigens as a means of fertility prevention. An embryonic antigen useful for fertility control should have the following characteristics: the antigen must be present on the embryonic surface in a density and for a length of time that would allow immunologic recognition and destruction of the cell; mediators of immune destruction should have access to the embryo that is unimpeded by physical barriers such as zona pellucida, decidual tissue or reproductive tract epithelium, and the embryonic target antigens should lack determinants found in adult tissues.

Alloantigens

Alloantigens are controlled by histocompatibility genes, and their expression in mouse embryos has been extensively studied.[19,20] In mice, the major histocompatibility (H-2) antigens are found on the embryonic cell surface in the early blastocyst stage, and the minor histocompatibility antigens may be expressed at the first cleavage stages.[21,22] However, murine alloantigens may be available for recognition only during the preimplantation periods, as their expression on the trophectoderm surface is reduced sharply with the initiation of implantation. This short period of expression and the highly polymorphic nature of alloantigens are felt to make them poor candidates for use in immunocontraception.[23]

Oncofetal Antigens

Oncofetal antigens have been detected on various spontaneous, chemically induced and virally transformed tumors. They are also detectable on embryonic cell surfaces.[24,25] F-9 teratocarcinoma oncofetal antigens are the most well studied of this group. In one study[26] female mice immunized with F-9

TABLE 1.—*Comparison of 'Ideal' Properties of Possible Antifertility Antigens*

	'Ideal' Properties					
Antigens	*High Antibody Titers*	*Persistence of Immunity*	*Possible Reversibility*	*Safe, Effective Adjuvant Available*	*Antibody Specificity*	*Free of Complications*
Zona pellucida	±	+	+	−	+	±
Spermatozoa						
LDH-C_4	−	+	±	−	+	±
Acrosin	−	+	±	−	+	±
Embryonic alloantigens	±	±	±	−	−	±
Oncofetal antigens	±	±	±	−	±	±
Structural trophoblastic antigens	±	±	±	−	±	±
Placental enzymes	−	−	−	−	−	−
Schwangershaft's protein-1	±	±	±	−	−	±
Placental protein-5	±	±	±	−	±	±
Human placental lactogen	±	−	±	−	−	−
Human chorionic thyrotropin	±	−	±	−	−	−
Human chorionic gonadotropin	±	±	±	−	+	±

LDH-C_4 = lactate dehydrogenase isoenzyme C_4, ± = equivocal, + = present, − = absent

cells showed a reduction in fertility. However, of three F-9 immunized mice that became pregnant, teratocarcinoma developed in one, and all fetuses of another were severely growth retarded.

Structural Trophoblastic Antigens

Because trophoblast is normally found only in cases of pregnancy, antigenic determinants unique to this tissue would offer potential sites for antibody binding. In mice it has been possible to obtain relatively pure trophoblast (called ectoplacental cone trophoblast) by dissecting the early postimplantation conceptus before infiltration of other tissues. It has thus been possible to raise a heterologous antiserum that shows specific binding to trophoblast cell populations.[23] The antigens differ from alloantigenic determinants in that they are not lost at the time of implantation. The difficulty with this approach for humans is obtaining sufficient quantities of trophoblast-specific antigens to effect immunization. Also, it is not known in humans whether or not antibodies to trophoblastic antigens would cross-react with other tissue antigens.

Placental Antigens

Placental proteins are unique to pregnancy and would provide logical targets for the action of an antifertility vaccine. The ideal antigen would be specific to early gestation, required for maintaining pregnancy and be present in trace amounts to facilitate neutralization by specific antibodies. A number of placental proteins have been characterized, including human placental lactogen (also known as human chorionic somatomammotropin), human chorionic thyrotropin, human chorionic gonadotropin (hCG), β_1-glycoproteins and placental enzymes.[27]

Placental enzymes. These include 17β- and 3β-hydroxysteroid dehydrogenases, cystine aminopeptidase and heat-stable alkaline phosphatase. However, these enzymes are not suitable candidates for an antifertility vaccine as they lack the requisite immunologic specificity.

β_1-Glycoproteins. Two human placental β_1-glycoproteins have been purified, Schwangershaft's protein-1 (SP_1) and placental protein-5 (PP_5), having molecular weights of 90,000 and 50,000, respectively.[27] Passive immunization of fertile female monkeys with anti-human SP_1 resulted in decreased fertility, although isoimmunization yielded less promising results.[28] Problems with using SP_1 as a basis for fertility control include the large amounts that are present in the placenta and its lack of tissue specificity.[29] On the other hand, PP_5 has been found to be a more potent antifertility agent than SP_1 and is capable of causing a significant reduction in fertility in monkeys immunized with this antigen.[27] However, the physiologic role of this glycoprotein during pregnancy has not been elucidated.

Human placental lactogen and human chorionic thyrotropin. Human placental lactogen (hPL) is secreted by the trophoblast and is detectable in serum at about the sixth gestational week. Although anti-hPL in serum was able to disrupt pregnancy in rats, mice and baboons, active immunization of baboons against baboon placental lactogen did not result in abortions.[27] Due to hPL's immunologic similarity to pituitary growth hormone, it would have to be suitably modified to avoid cross-reactivity. Human chorionic thyrotropin, like hPL, has considerable immunologic cross-reactivity with its pituitary counterpart and major molecular modifications would have to be made to obviate this problem.

Human Chorionic Gonadotropin

Human chorionic gonadotropin has received much attention as a target for an antifertility vaccine due to its unique physiologic role in human reproduction. This hormone is normally produced only during pregnancy and is not present in nonpregnant women. After implantation, hCG is secreted by the trophoblast into the maternal circulation, rescues the corpus luteum from degeneration and stimulates it to secrete estrogen and progesterone.[30,31] Not until placental steroid production surpasses that of the corpus luteum at the ninth gestational week does the corpus luteum become unnecessary for endometrial support. hCG thus bridges the transition from ovarian to placental steroid function. Because hCG travels via the maternal circulation from the trophoblast to the corpus luteum, it is subject to inactivation by specific circulatory antibodies. It has been postulated that serum antibodies with a binding capacity of 20 ng of hCG per ml of serum would be sufficient to interrupt pregnancy.[32]

Whether hCG is secreted by the blastocyst before or after implantation has yet to be resolved. Using a radioreceptor assay that detects both hCG and human luteinizing hormone (LH), Saxena and co-workers found increased levels of hCG as early as six days after ovulation.[33] Other investigators, however, did not detect a significant rise in plasma hCG until 9 to 13 days following ovulation, precluding the likelihood that hCG is secreted before implantation of the blastocyst.[34] Regardless of the chronology of hCG secretion, it is of paramount importance during the first trimester of pregnancy.

Human chorionic gonadotropin, a glycoprotein of molecular weight 39,000, is composed of an α- and a β-subunit.[35] The β-subunit, with a molecular weight of 23,000,[36] is responsible for the hormonal identity of hCG.[37] The structure of hCG is similar to that of the pituitary glycoproteins, thyroid-stimulating hormone (thyrotropin), follicle-stimulating hormone (FSH) and luteinizing hormone. As in the case of hCG,the β-subunit of these pituitary hormones confers to each its own biologic specificity. The α-subunits of hCG, LH, FSH and thyrotropin are nearly identical.[37] However, β-hCG has an additional 30 amino acid residues at its carboxyterminus that are not found in the β-subunits of the other glycoprotein hormones, with four of the additional serine residues possessing carbohydrate side chains.[38] Due to the similar amino acid sequences of hCG and human LH, use of the former as an antigen would necessitate that it be highly purified; otherwise a high cross-reactivity with human LH would exist.

The considerable amino acid homology between the β-subunits of hCG and human LH and the low molecular weight of β-hCG promoted studies to increase its specific antigenic qualities. These included (1) coupling the subunit to a protein carrier[39]; (2) using enzyme-cleaved fragments from the unique COOH-terminus of the β-subunit[40]; (3) constructing synthetic peptides with amino acid structures similar to the COOH-terminal region[41]; (4) using synthetic COOH-terminal peptide conjugated to antigenic carrier proteins,[42] and (5) chemical modification of the β-subunit with specific functional reagents.[27] The rationale for these approaches was supported by reports that indicate that antibodies against β-hCG could discriminate hCG from human LH.[43,44]

Spatial configuration of the molecule also plays an important role in the antigenicity of β-hCG. Partial reduction and s-carboxamidomethylation of the β-subunit induces a change in its three-dimensional structure that leads to preferential loss of human LH and retention of hCG immunoactivity.[45] Lerario and associates found that hCG-specific determinants, which reside in the variable region of the β-subunit, are predominantly conformational rather than sequential in nature.[46] They also found that antigenic determinants, usually buried when β-hCG is combined with its complementary α-subunit, are subsequently uncovered when β-hCG dissociates. Antibodies produced against the dissociated β-subunit were not as sensitive as antisera to the endogenous intact hormone, or the β-hCG coupled to a nonhuman α-subunit.

Stevens and colleagues used β-hCG hapten coupled with *p*-aminobenzene-sulfonic acid to immunize baboons.[27] The antibodies produced showed cross-reactivity with human but only weakly with baboon LH. Talwar and co-workers showed that injection of β-hCG coupled with tetanus toxoid (β-hCG-TT) elicited a desirable antibody response to the native hormone in animals.[47] They reported that anti-β-hCG-TT sera raised in various species neutralized the biologic activity of hCG in three of four test systems. Additional work showed that preimmunization with tetanus toxoid carrier would enhance neutralizing antibody production to β-hCG.[48] Studies in rhesus monkeys showed that hCG administered after immunization with β-hCG-TT resulted in a decline in circulating antibody titers. Therefore, these antibodies were able to recognize and bind the hCG molecule.[49] When human LH was administered in lieu of hCG, there was no detectable decline in anti-hCG titer. Native hCG did not act as a booster to induce an anamnestic response.[5]

Bahl and co-workers have developed a highly specific derivative of β-hCG (DS_5-hCG-β-hem) by the controlled reduction and alkylation of disulfide bonds followed by conjugation to keyhole limpet hemocyanin. Rabbit antibody to DS_5-hCG-β-hem specifically bound hCG with no significant cross-reactivity with human LH and other polypeptide hormones.[27] The antibody neutralized in vivo the effect of hCG on ovarian ascorbic acid content without affecting human LH activity. It also inhibited hCG-induced rat uterine weight gain.

To date, only β-hCG-TT has been tested in phase I clinical trials; 15 young adult women who had previously undergone tubal ligation were immunized with this conjugate.[32] Antibodies developed in 14 of the women, and no adverse effects were noted. Unfortunately, sufficiently high anti-hCG titers to ensure adequate binding of maternal hCG did not develop. Interestingly, high antibody titers against the tetanus toxoid carrier protein were produced. This discrepancy in antibody response to the two components of the conjugate could be explained by the following: β-human chorionic gonadotropin is an isoantigen and therefore not a potent immunogen in its own right; because the tetanus toxoid carrier contains polymers with molecular weights 100 times greater than that of β-hCG, its large size may impede recognition of the hapten, resulting in decreased antibody production; and the use of a bifunctional reagent might join not only hapten to carrier, but also β-hCG to β-hCG and carrier to carrier, thus reducing the net dose of immunogenic polymer.

Additional human trials have been conducted to further evaluate β-hCG-TT. Recently 23 women were immunized with this conjugate, but peak titers could not be sustained and most showed a variable pattern of antibody response.[50]

Although not of placental origin, the β-subunit of the ovine pituitary glycoprotein, luteinizing hormone (OLHβ), is able to elicit production of antibodies in the serum of rhesus monkeys that cross-react strongly with rhesus chorionic gonadotropin with resultant reduced fertility.[51] The antifertility effect of anti-OLHβ is probably due to prevention of corpus luteum rescue.[52] Anti-OLHβ was also able to bind hCG,[51] which leads to the possible extrapolation of this technique to humans.

Adjuvants

The problem of sufficient antibody production to any reproductive tract immunogen probably also extends to the type of adjuvant used. Although the use of alum-precipitated antigen has proved to be a safe vehicle for human vaccination, it stimulates insufficient antibody response to β-hCG or other isoantigens. Talwar points out that unless Freund's complete adjuvant is used, investigators have been unsuccessful in stimulating antibodies to synthetic fragments of β-hCG.[53] Freund's complete adjuvant, although a potent adjuvant, causes extensive and disfiguring granuloma formation and therefore is not acceptable for human use.

To achieve a state of enhanced immune responsiveness to a putative reproductive tract antigen, an adjuvant may act in one or more of three ways.[54] First, a slow release of antigen from the site of deposition may prolong and thereby potentiate the antigenic stimulus. Second, the adjuvant could stimulate the mononuclear phagocyte system, causing rapid ingestion or more effective processing of antigen and enhanced lymphocytic response. Third, adjuvants might evoke a generalized immune response, such as a direct action on lymphocytes or an acceleration of cellular differentiation.

A number of adjuvants have been tested for possible use with an antifertility vaccine. Muramyl dipeptide or one of its analogs has recently been shown to be a highly effective adjuvant. It has the following advantages: it is a simple synthetic compound with many analogs, it is soluble in water, it is not cytotoxic and it is not antigenic in its own right.[55] The use of muramyl dipeptide with β-hCG-TT is in the initial stages of investigation.[56] Also, Covey and associates have shown that *Corynebacterium parvum* may potentiate neutralizing antibody production to β-hCG-TT.[57] Other adjuvants considered include thymic factor, lavamisole, tilorone, liposomes and *Bordetella pertussis*. Perhaps one of the foregoing adjuvants or a new immunoenhancing compound such as lynestrenol[58] will provide the breakthrough needed to produce a safe, effective vaccine.

Potential Complications of Antifertility Vaccines

Complications Associated With Active Immunization

Immunization may result in anaphylactic reactions mediated by mast-cell-bound IgE antibodies. Factors that predispose a person to such reactions include a small antigenic dose[59]; genetic susceptibility[60]; the use of certain adjuvants, especially pertussis,[61,62] and IgA deficiency.[63] Determining the presence of antigen-specific serum IgE is possible by the radioallergosorbent test and may be helpful in averting this potentially life-threatening problem. Other complications of

active immunizations include delayed hypersensitivity and Arthus reactions.

Possible Complications Associated With hCG or hCG-Subunit Immunizations

Because the α-subunit of hCG is nearly identical to the α-subunits of thyrotropin, FSH and LH, immunization with the intact hCG molecule could cause production of antibodies that cross-react with these other hormones.[36] These cross-reactive antibodies could then neutralize LH, FSH and thyrotropin and cause decreased function of the hormones' target organs. Immunization with the β-subunit of hCG would avoid the production of antibodies that cross-react with thyrotropin and FSH. However, the β-subunits of LH and hCG are very similar in structure and the potential for formation of antibody that reacts with LH would still exist with β-hCG immunization.

Immunization with pituitary hormones or pituitary hormone analogs may damage the pituitary gland itself. There is indirect evidence that antibodies to pituitary hormones may damage the pituitary gland, either via direct cytotoxicity[64,65] or by forming insoluble complexes on the cell surface and eliciting an Arthus reaction.[66]

Serum sickness is another potential complication of hCG immunization because cross-reactive antibodies could also form circulating immune complexes with pituitary hormones. However, because circulating immune complexes most readily form in situations of slight antigen excess,[67] the extremely low circulating levels of thyrotropin, FSH and LH make this complication unlikely.

Finally, because human malignant tumors may express hCG or embryonic antigens on their surfaces, it has been postulated[68] that masking of these surface antigens by non-complement-fixing antibodies could impede T-lymphocyte recognition and destruction—that is, immunologic surveillance. Also, Hurtenbach and Shearer[69] have shown that immunizing mice with sperm antigens impairs cancer immunosurveillance through generation of T-suppressor lymphocytes.

Possible Complications Associated With Sperm Antigen Immunizations

In 1966 Paterson[70] reported that in Lewis rats immunized with testicular antigens, pathologic changes developed in their brains that were histologically consistent with the findings in cases of allergic encephalomyelitis. However, the possibility that the rats were infected with viral encephalomyelitis, a common disease in Lewis rats, was not excluded.[68]

Other studies have shown the existence of cross-reactive antigens found in both mouse sperm and fetal tissue.[68,71] This raises the possibility that antisperm antibodies could cross-react with fetal tissue and harm or modify fetal development. Cross-reactive antisperm antibodies might also interfere with immunologic surveillance of fetal-antigen-secreting tumors.

Finally, Tung[68] has reported that amyloidosis has developed in sperm-immunized guinea pigs. Therefore, he has raised the question of whether widespread amyloidosis could occur in humans immunized with sperm antigens.

Conclusion

For an antifertility vaccine to be efficacious, it must cause an adequate antibody titer even in poor responders. In this regard, adjuvant research looks to improve these titers. Both natural and synthetic adjuvants are being tested. It is estimated that another 10 to 15 years of sustained, high-priority effort will be needed to produce a workable vaccine using either β-hCG or one of the other compounds.[72] An effective antifertility vaccine should become a reality when the right match of antigen and adjuvant is discovered.

REFERENCES

1. Hanada A, Chang MC: Penetration of zona free eggs by spermatazoa of different species. Biol Reprod 1972 Apr; 6:300-309
2. Barros C, Yanagamachi R: Polyspermy preventing mechanisms in the golden hamster eggs. J Exp Zool 1972 May; 180:251-266
3. Sacco AG: Immunocontraception: Consideration of the zona pellucida as a target antigen, *In* Wynn RM (Ed): Obstetrics and Gynecology Annual, Vol 10. New York, Appleton-Century-Crofts, 1981, pp 1-26
4. Dunbar BS, Raynor BD: Isolation and characterization of porcine zona pellucida antigens. Fed Proc 1979 Mar 1; 38:446
5. Talwar GP: Immunology in reproduction. J Reprod Med 1979 Feb; 22:61-76
6. Tsunoda Y: Inhibitory effect of anti-mouse egg serum on fertilization in vitro and in vivo in the mouse. J Reprod Fertil 1977 Jul; 50:353-355
7. Shivers CA, Dudkiewicz AB, Franklin LE, et al: Inhibition of sperm-egg interaction by specific antibody. Science 1972 Dec 15; 178:1211-1213
8. Takai I, Mori T, Noda Y, et al: Heteroimmunization with isolated human oocytes. J Reprod Fertil 1981 Jan; 61:19-24
9. Sacco AG, Yurewicz EC, Subramanian MG, et al: Zona pellucida composition: Species cross-reactivity and contraceptive potential of antiserum to a purified pig zona antigen (PPZA). Biol Reprod 1981 Dec; 25:997-1008
10. Goldberg E: Amino acid composition and properties of crystalline lactate dehydrogenase-X from mouse testes. J Biol Chem 1972 Apr; 247:2044-2048
11. Goldberg E: Infertility in female rabbits immunized with lactate dehydrogenase-X. Science 1973 Aug 3; 181:458-459
12. Goldberg E: Sperm specific antigens and immunological approaches for control of fertility, *In* Talwar GP (Ed): Recent Advances in Reproduction and Regulation of Fertility. Amsterdam, Elsevier/North-Holland, 1979, pp 281-290
13. Stambaugh R, Buckley J: Identification and subcellular localization of the enzymes effecting penetration of the zona pellucida by rabbit spermatozoa. J Reprod Fertil 1969 Aug; 19:423-432
14. Meizel S, Lui CW: Evidence for the role of a trypsinlike enzyme in the hamster sperm acrosome reaction. J Exp Zool 1976 Jan; 195:137-144
15. Stambaugh R, Brackett BG, Mastroianni L Jr: Inhibition of *in vitro* fertilization of rabbit ova by trypsin inhibitors. Biol Reprod 1969 Sep; 1:223-227
16. Zaneveld LJD, Robertson RT, Kessler M, et al: Inhibition of fertilization *in vivo* by pancreatic and seminal plasma trypsin inhibitors. J Reprod Fertil 1971 Jun; 25:387-392
17. Beyler SA, Zaneveld LJ: Antifertility activity of systemically administered proteinase (acrosin) inhibitors. Contraception 1982 Aug; 26:137-146
18. Morton DB: Lysosomal enzymes in mammalian spermatozoa, *In* Dingle TT, Dean RT (Eds): Lysosomes in Biology and Pathology, Vol 5. Amsterdam, Elsevier/North-Holland, 1976, pp 203-255
19. Jenkinson EJ, Billington WD: Cell surface properties of early mammalian embryos, *In* Sherman MI (Ed): Concepts in Mammalian Embryogenesis. Cambridge, Mass, MIT Press, 1977, pp 235-266
20. Muggleton-Harris AL, Johnson MH: The nature and distribution of serologically detectable alloantigens on the preimplantation mouse embryo. J Embryol Exp Morphol 1976 Feb; 35:59-72
21. Håkansson S, Heyner-Joshi S, Sundqvist KG, et al: The presence of paternal H-2 antigens on hybrid mouse blastocysts during experimental delay of implantation and the disappearance of these antigens after onset implantation. Int J Fertil 1975 Jul-Sep; 20:137-140
22. Searle RF, Sellens MH, Elson J, et al: Detection of alloantigens during preimplantation development and early trophoblast differentiation in the mouse by immunoperoxidase labeling. J Exp Med 1976 Feb; 143:348-359
23. Billington WD: Immunological interference with implantation. Upsala J Med Sci 1978; 22(suppl):51-58
24. Allison AC: Antigens shared by tumour cells and foetal or gonadal cells. Clin Exp Immunoreprod 1975; 1:19-41
25. Baldwin RW, Vose BM: The expression of a phase-specific foetal antigen on rat embryo cells. Transplantation 1974 Dec; 18:535-530
26. Hamilton MS, Beer AE, May RD, et al: The influence of immunization of female mice with F-9 teratocarcinoma cells on their reproductive performance. Transplant Proc 1979 Mar; 9:1060-1072
27. Bahl OP, Muralidhar K: Current status of antifertility vaccine, *In* Dhindsa DS, Schumacher GFB (Eds): Immunological Aspects of Infertility and Fertility Regulation. New York, Elsevier/North-Holland, 1980, pp 225-257
28. Stevens VC: Immunological approaches to fertility regulation. Bull WHO 1978; 66:179-191
29. Searle F, Bagshawe KD, Leake BA, et al: Serum-SP_1-pregnancy-specific-β-glycoprotein in choriocarcinoma and other neoplastic disease. Lancet 1978 Mar 18; 1:579-580
30. Vande Wiele RL, Bogumil J, Dyrenfurth I, et al: Mechanisms on regulating the menstrual cycle in women. Recent Prog Horm Res 1970; 26:63-95
31. Canfield RE, Birken S, Morse JH, et al: Human chorionic gonadotropin, *In* Parsona JA (Ed): Peptide Hormones. Baltimore, University Park Press, 1976, pp 299-315

32. Nash H, Talwar GP, Segal S, et al: Observations on the antigenicity and clinical effects of a candidate antipregnancy vaccine: β-Subunit of human chorionic gonadotropin linked to tetanus toxoid. Fertil Steril 1980 Oct; 34:328-335

33. Saxena BB, Hasan SH, Haour H, et al: Radioreceptor assay of human chorionic gonadotropin: Detection of early pregnancy. Science 1974 May 17; 184:793-795

34. Catt KJ, Dufau ML, Vaitukaitis JL: Appearance of hCG in pregnancy plasma following the initiation of implantation of the blastocyst. J Clin Endocrinol Metab 1975 Mar; 40:537-540

35. Morgan FJ, Birken S, Canfield RE: Chemistry of human chorionic gonadotropin, *In* Moudgal NR (Ed): Gonadotropins and Gonadal Function. New York, Academic Press, 1974, pp 79-92

36. Carlson RB, Bahl OP, Swaminsathan N: Human chorionic gonadotropin: Linear amino acid sequence of the β-subunit. J Biol Chem 1973 Oct 10; 248:6810-6825

37. Pierce JG: The subunits of pituitary thyrotropin: Their relationship to other glycoprotein hormones. Endocrinology 1971 Dec; 89:1331-1344

38. Morgan FJ, Birken S, Canfield RE: Human chorionic gonadotropin: A proposal for the amino acid sequence (Letter). Mol Cell Biochem 1973 Nov; 2:97-99

39. Talwar GP: Immunological approaches to fertility control, *In* Diezfalusy E (Ed): 7th Karolinska Symposium. Stockholm, Karolinska Institut, 1974, p 370

40. Chen HC, Hodgen GD, Matsura S, et al: Evidence for a gonadotropin from non-pregnant subjects that has physical immunological and biological similarities to hCG. Proc Natl Acad Sci USA 1976; 73:2885-2889

41. Matsura S, Ohashi M, Chen HC, et al: An hCG specific antiserum against synthetic peptide analogs to the C-terminal peptide of its β-subunit. Endocrinology 1979 Feb; 104:396-401

42. Ramakrishnan S, Das C, Dubey SK, et al: Immunogenicity of three C-terminal synthetic peptides of the β-subunit of human chorionic gonadotropin and properties of the antibodies raised against 45 amino acid C-terminal peptide. J Reprod Immunol 1979 Dec; 1:249-261

43. Vaitukaitis JL, Braunstein GD, Ross GT: A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. Am J Obstet Gynecol 1972 Jul; 113:751-758

44. Salahuddin M, Ramakrishnan S, Dubey SK, et al: Immunological reactivity of antibodies produced by Pr-β-HCG-TT with different hormones. Contraception 1976 Feb; 13:163-170

45. Bahl OP, Pandian MR, Ghal RD: Immunological properties of the beta-subunit of human chorionic gonadotropin. Biochem Biophys Res Commun 1976 May; 70:525-532

46. Lerario AC, Pierce JG, Vaitukaitis JL: Effect of conformation of hCG-beta on generation of hCG-specific antibody. Endocrin Res Commun 1978 Jan; 5:43-55

47. Entire issue. Contraception 1976 Feb; 13:128-268

48. Covey DC, Laurence KA: Effect of carrier preimmunization on neutralizing antibody response of rabbits immunized with β-human chorionic gonadotropin coupled to tetanus toxoid. Contraception 1982 Dec; 26:595-606

49. Ramakrishnan S, Das C, Talwar GP: Nature of immune complexes in rhesus monkeys immunized with Pr-β-hCG-TT on challenge with hCG. Contraception 1978 Jul; 18:71-90

50. Shahani SM, Kulkarni PP, Patel KL, et al: Clinical and immunological responses with Pr-β-hCG-TT vaccine. Contraception 1982 Apr; 25:421-434

51. Sundaram K, Chang CC, Laurence KA, et al: The effectiveness in rhesus monkeys of an antifertility vaccine based on neutralization of chorionic gonadotropin. Contraception 1976 Sep; 14:639-653

52. Thau RB, Sundaram K: The mechanism of action of an antifertility vaccine in the rhesus monkey: Reversal of the effects of antisera to the β-subunit of ovine luteinizing hormone by medroxyprogesterone acetate. Fertil Steril 1980 Mar; 33:317-320

53. Talwar GP: Immunology in reproduction. J Reprod Med 1979 Feb; 22:61-76

54. Dresser DW, Phillips JM: The cellular targets for the action of adjuvants: T-adjuvants and B-adjuvants, *In* Immunopotentiation: Proceedings—Ciba Foundation Symposium No. 18. Amsterdam, Elsevier/North-Holland, 1973, pp 3-18

55. Tanaka A, Nagao S, Nagao R, et al: Stimulation of the reticuloendothelial system of mice by muramyl dipeptide. Infect Immun 1979 May; 24:302-307

56. Gonzalez ER: Contraceptive vaccine research still an art. JAMA 1980 Sep 26; 244:1414-1419

57. Covey DC, Eddy EM, Laurence KA: The role of human chorionic gonadotropin in contraceptive vaccine research. Biologist 1982 Aug; 64:49-57

58. Wybran J: Lynestrenol: A progesterone-like agent with immunostimulatory properties. Recent Results Cancer Res 1980; 75:180-184

59. Levine BB, Vaz NM: Effect of combination of inbred strain, antigen and antigen dose on immune responsiveness and reagin production in the mouse—A potential mouse model for immune aspects of human atopic allergy. Int Arch Allergy Appl Immunol 1970; 39:156-171

60. Levine BB, Stember RH, Fotino M: Ragweed hay fever: Genetic control and linkage to HL-A haplotypes. Science 1972 Dec 15; 178:1201-1203

61. Mota I: The mechanisms of anaphylaxis—I. Production and biologic properties of mast cell sensitizing antibody. Immunology 1964 Nov; 7:681-699

62. Binaghi RA, Benacerraf B, Bloch KJ, et al: Properties of rat anaphylactic antibody. J Immunol 1964 Jun; 92:927-933

63. Polmar SH, Waldmann TA, Balestra ST, et al: Immunoglobulin E in immunologic deficiency diseases—I. Relation of IgE and IgA to respiratory tract disease in isolated IgE deficiency, IgA deficiency and ataxia telangiectasia. J Clin Invest 1972 Feb; 51:326-330

64. Goudie RB, Pinkerton PH: Anterior hypophysitis and Hashimoto's disease in a young woman. J Pathol Bacteriol 1962 Apr; 83:584-585

65. Bottazzo GF, Florin-Christensen A, Pouplard A, et al: Autoantibodies to prolactin-secreting cells of human pituitary. Lancet 1975 Jul 19; 2:97-101

66. Clagett JA, Wilson LB, Weigle WO: Interstitial immune complex thyroiditis in mice—The role of autoantibody to thyroglobulin. J Exp Med 1974 Dec; 140:1439-1456

67. Disease of Immunity—Disorders of the Immune System, *In* Robbins SR, Cotran RS (Eds): Pathologic Basis of Disease, 2nd Ed. Philadelphia, WB Saunders, 1979, p 285

68. Tung KSK: Antifertility vaccines: Considerations of their potential immunopathologic complications. Int J Fertil 1976 Oct-Dec; 21:197-206

69. Hurtenbach U, Shearer GM: Germ cell-induced immune suppression in mice: Effect of inoculation of syngeneic spermatozoa on cell-mediated immune responses. J Exp Med 1982 Jun; 155:1719-1729

70. Paterson PY: Experimental allergic encephalomyelitis and autoimmune disease. Adv Immunol 1966; 5:131-208

71. Artzt K, Dubois P, Bennett D, et al: Surface antigens common to mouse cleavage embryos and primitive teratocarcinoma cells in culture. Proc Natl Acad Sci USA 1973 Oct; 70:2988-2992

72. Atkinson L, Shearer SB, Harkavy O, et al: Prospects for improved contraception. Fam Plann Perspect 1982 Jul-Aug; 14:173-192